

# COMPARISON OF STEAM AND HOT LIQUID WATER PRETREATMENTS FOR THE BIOCONVERSION OF LIGNOCELLULOSES TO ETHANOL

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## INTRODUCTION

In order for lignocellulosic substrates to be made biologically available to microorganisms, which convert them to ethanol, these materials must first be pretreated. Since pretreatment is one of the most costly steps in ethanol production, accounting for 33% of total processing cost according to a base case design by the National Renewable Energy Laboratory (NREL - Lynd, et. al., 1996), it has been widely investigated. The bulk of pretreatment research has focused on acid and steam pretreatment of the lignocellulose. However, these methods may pose difficulties associated with material recovery, inhibitor formation, and waste disposal.

Successful pretreatment methods are known to disrupt cells and open the lignocellulose matrix (Kallavus and Gravitis, 1995), increasing pore volume (Gretlein and Converse, 1991; Thompson et. al. 1992) and available surface area (Thompson et. al. 1992). There is also a correlation between hemicellulose solubility and pretreatment effectiveness. Lignin is chemically modified during pretreatment. It has a melting temperature lower than typical pretreatment temperatures and does not return to its initial state (Torget et. al., 1991; Ooshima, et. al., 1990). High hydrogen ion concentrations accelerate sugar degradation (Leesomboon, 1988 & Antal et. al., 1991) and maintaining neutral pHs has been shown to improve pentosan recoveries (Weil et. al. 1998).

Water seems to be an important contributor to the pretreatment process. Due to the increased disproportionation of water at high temperatures, water lowers the pH of the reaction environment. The ion product concentration of liquid water at 220 °C is  $10^{-11}$  and results in a pH of 5.6 compared to 7.0 the pH of water at 25°C (Marshall & Franck, 1981). Hydrolytic reactions cleave glycosidic linkages in hemicellulose and lignin. There is also evidence for the elementary bimolecular cleavage of the ether bond by water at 225°C (Xu et. al. 1997). Additionally, water lowers the softening point of lignin (Goring, 1963). Hemicellulose is deacetylated by water (Bouchard, et. al., 1991) and both lignin and hemicellulose are depolymerised by water (Bobbleter and Concinn, 1979 and Chua and Wayman, 1979).

The use of water at elevated temperature and pressure to fractionate biomass into its constituents was first employed in the 1930s (Aronovsky and Gortner, 1930). Recently, however, it has received renewed recognition as a possible pretreatment for ethanol production (Bobbleter and Concinn, 1979, van Walsum et. al. 1996, & Weil et. al. 1998). Mok and Antal (1992) were able to completely remove hemicellulose from hardwoods and herbaceous material, without significant degradation. More recently, pretreatment with hot liquid water at 220°C and up to 2 minutes has been shown to be extremely effective for both fractionation and bioconversion (Allen et. al. 1996 and Van Walsum et. al. 1996).

A complete mechanistic understanding as to why pretreatment is effective at rendering lignocellulose amenable to enzymatic attack has remained illusive. Due to this uncertainty empirical evidence is used for process optimization. Pretreatment effectiveness and cost can be rated based upon several metrics. These include reactivity, pentosan recovery, inhibitor formation, solubilization, feed particle size reduction, materials of equipment construction, and residues. However, care must be taken to identify factors that might increase costs down the line.

In this paper we focus on the effectiveness of pretreatment methods as measured by their solubilization, pentosan recoveries, inhibitor formation, and solids concentration. Solubilization is a measure of fractionation of recalcitrant crystalline cellulose from the less ordered hemicellulose and lignin, which more readily enters the liquid fraction. Greater solubilization results in more accessible pore areas in the lignocellulosic residue (LCR) for bioconversion (Weil et. al., 1994), and separates out the hemicellulose and lignin. Under pretreatment conditions, which typically produce reactive cellulose, xylose is easily degraded. Thus, it is not surprising that many pretreatment methods degrade xylose. For example, xylose recoveries with steam pretreatment are at best 65% (Heitz et. al. 1991). Technology has recently become available which allows for bioconversion of pentosan sugars to ethanol. In order for enzymatic bioconversion of biomass to ethanol to be economically competitive with acid processes and gasoline, it is necessary to get the most from the feed and thus xylose recovery is an extremely important factor along with a minimization of hydrolyzate inhibition products. Therefore, an understanding of inhibitor formation and an optimization of conditions designed to reduce their formation are necessary. Furfural, a breakdown product of xylose, is a known inhibitor of cellulase enzymes. It can be used as a measure of inhibitor formation. Many of the products formed by xylose degradation are cellulose inhibitors. Therefore furfural is a good gauge of the amount of inhibitors. Solids concentration is important because dilute process streams are undesirable, increasing process costs.

The results of steam pretreatment (SP) and hot water pretreatment (HLWP) liquid are presented in this paper of sugarcane bagasse. These pretreatment techniques were compared on the basis of metrics including pentosan recovery, hydrolyzate inhibitor formation, and solubilization. Emphasis was placed on understanding the inherent chemical differences between the techniques.

## MATERIALS AND METHODS

Sugarcane bagasse was used as a representative feedstock in order to compare SP and HLWP techniques. A custom-built aqueous fractionation device was employed which could pretreat up to 1 kg of biomass (50 % moisture) as a batch using hot liquid water, steam or both. Details of the reactor appear in Allen et. al. (1998). Pretreatment by HLW was preceded by a 20-50s steam preheat of the material in order that latent heat could be used to arrive at the desired temperatures and high transient temperatures could be avoided. Timing began after addition of the water was completed for HLWP and after the desired temperature was attained for SP. After the reaction the LCR was rinsed with cold water and this fluid, the flush, was collected. Feed material was pretreated with both techniques for 2-5 minutes at 220 °C.

The analytical procedures used to characterize the feed material, lignocellulosic residues and liquid products are also described in Allen et. al. 1998 and are based on NREL's Ethanol Project LAP 002 & 014 (Ruiz and Ehrman, 1996). The amount of solubilized material present in the extract was determined by evaporating duplicate aliquots (20 to 25 g each) to dryness (1 d @ 105 °C).

## RESULTS AND DISCUSSION

Results of experiments, comparing aqueous fractionation of sugarcane bagasse with steam and HLW appear in Table I. All experiments were performed at 220°C with 2 and 5-minute reaction times.

Approximately 35% solubilization was achieved for a 2-minute reaction time with both pretreatment modes. These values are not as high as those obtained by Allen et al. (1996) for HLW (50%), but higher than the 30% solubilization achieved with steam pretreatment by Jollez et al. (1994). For a reaction time of 5 minutes, solubilization increased for HLWP (44%), but decreased for SP. The decrease in solubilization with time associated with SP can be attributed to the formation of insoluble breakdown products (tars and resins) from solubilized xylose. This hypothesis is supported by the observation of a dark, tar-like sticky coating, which was soluble in acetone, and coated the lignocellulosic residue (LCR) for the 5-minute steam experiment. In the chemistry of xylose breakdown, under the conditions being employed, there are many higher order reactions, which are not well understood, but which result in resin formation (Leesomboon, 1988).

Significantly higher solids concentration were achieved with SP as compared to pretreatment with HLW. These experiments did not focus on achieving high solids concentration and with optimization, higher solids concentrations could be achieved for HLW, however, due to the nature of the techniques, these values will never approach those achievable with SP. It is therefore unclear whether, in analyzing these experiments we are looking at SP versus HLW or high solids versus low solids aqueous pretreatment. A low solids concentration can result in increased costs associated with both the energy requirements to heat the greater amount of required water and to process a dilute extract.

From the xylose recovery data, it is apparent that xylose degradation is occurring. In all cases, xylose recovery decreases with increasing time (figure 1). This is understandable, since an increase in time gives more time for the formation of products, which turn around and attack the parent via higher order reactions. For both reaction times, SP results in lower xylose recoveries and more inhibition product formation (table 2). Furfural concentration increases with decreasing pentosan recovery and increasing time (figure2). Its yield from xylose breakdown is approximately 20-30 mol % at 250 °C (Antal et. al., 1991). Therefore, it is not expected to account for all lost xylose, but does indicate degradation and inhibitor formation.

With HLWP, the LCR is immersed in water. Therefore, when the hemicellulose is solubilized it will dissolve in the extract liquid. Since water has a lower boiling point than dissolved products of hemicellulose, the vapor above the mixture should be mostly water and, as observed, most of the solubilized hemicellulose was recovered in the extract. In SP, in order for this material to be collected as extract, it must either enter the vapor phase and condense or dissolve in the steam condensate, which condenses on the LCR and reactor walls and drips down. This is the reason that after SP experiments, the flush contains most of the dissolved hemicellulose products. This statement does not hold true for furfural and acetic acid since they have low vaporization temperatures, 162°C and 118°C respectively, and can easily enter the vapor phase and recondense to form extract.

SP solubilized material is more likely to enter the vapor phase than in HLWP because it is extremely concentrated in the liquid phase. This can lead to decreased recoveries associated with recondensation and vapor losses. If steam, containing dissolved xylose oligomers and its breakdown products, is released from the reactor, these compounds may be lost with the steam.

As furfural enriched steam cools, the possibility of loss of vapor phase products due to recondensation on the reactor and tank walls is increased.

Another reason for the xylose losses associated with steam pretreatment is losses associated with the formation of breakdown products. With SP, pentosan recovery, hydrolyzate inhibition and solubilization are interdependent. As xylose is broken down, inhibition products are formed. This leads to decreases in xylose recoveries and solubilization, since as the products are formed soluble xylose is converted into insoluble resins, which are difficult to analyze and detect. The material balances reinforce this concept, since they indicate limited xylose loss. As mentioned earlier, resins were visible on the LCR and if the yield of furfural from xylose is 20-30 mol %, degradation is significant in SP.

In aqueous chemistry, the water acts as a buffer. All products, which dissolve in the water, are diluted. This will affect the pH of the reaction, since acetic acid is present due to deacetylation of hemicellulose and lactic and formic acids are present, as a result of xylose degradation. In steam pretreatment, where there is a very small amount of condensed liquid on the surface of reacting material, acids will be concentrated in this liquid phase, resulting in a high  $[H^+]$  concentration. This concentration influences xylose breakdown, since acid catalyzes the xylose breakdown reaction. Leesomboon (1988) found that reaction rate, for a given time and temperature, depends solely on  $[H^+]$  concentration.

## CONCLUSIONS

Results indicate that HLWP performed better than SP on the basis of sugar recovery, hydrolyzate inhibitor formation and solubilization. Explanations, accounting for these differences are related to the different thermochemical conditions, which the feed material encounters. These conditions can lead to vapor losses, losses associated with recondensation, and carbohydrate breakdown. Additionally, shorter reaction times resulted in less xylose degradation and furfural formation. SP performed better on the basis of solids concentration.

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Table 1: Summary of experimental results

Reaction Medium	Time min.	Solids Concentration <sup>1</sup> %	Glucose Recovery %	Xylose Recovery %	Solubilization %	Material Balance %
Steam	2	18.3	98	48	35	87
	5	34.3	104	25	20	86
Hot Liquid Wat	2	2.4	103	83	36	97
	5	1.4	98	68	43	91

<sup>1</sup>Solids concentration is the mass of dry feed material expressed as a percentage of the total liquid in which it is immersed (extract + LCR moisture).

<sup>2</sup>Solubilization is calculated as dry LCR/ dry feed x 100

Table 2: Carbohydrate recoveries and degradation product formation

Reaction Medium	Time (min.)	Glucose Recovery (%)	Xylose Recovery (%)	Furfural (g/g Feed Xylose)
Steam Experiments				
Extract <sup>1</sup>	2	0	0	5
Flush <sup>2</sup>		4	36	0
LCR <sup>3</sup>		93	12	
Extract	5	0	0	5
Flush		1	5	12
LCR		103	21	
Hot Liquid Water				
Extract	2	3	63	3
Flush		0	5	0
LCR		100	15	
Extract	5	3	58	4
Flush		0	2	0
LCR		95	8	

<sup>1</sup> Extract - the liquid which is collected out of the bottom of the reactor after an experiment

<sup>2</sup> Flush - the cold water with which the hot LCR is rinsed after the reaction.

<sup>3</sup> LCR - lignocellulosic residue, the material which remains as a solid after pretreatment.

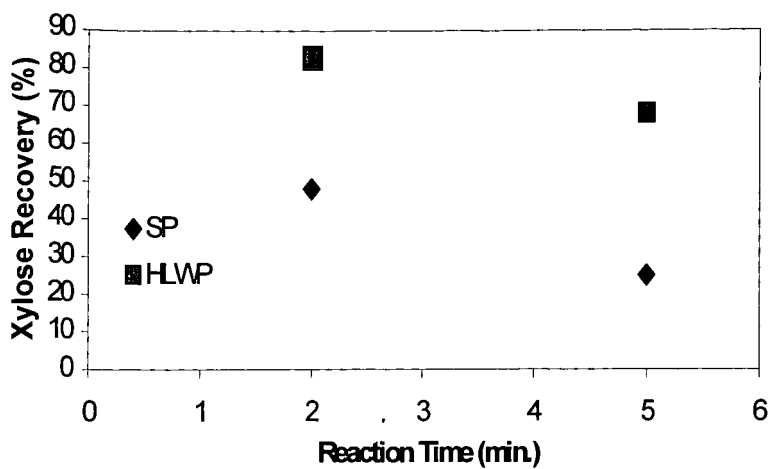


Figure 1: Xylose recovery as a function of reaction time.

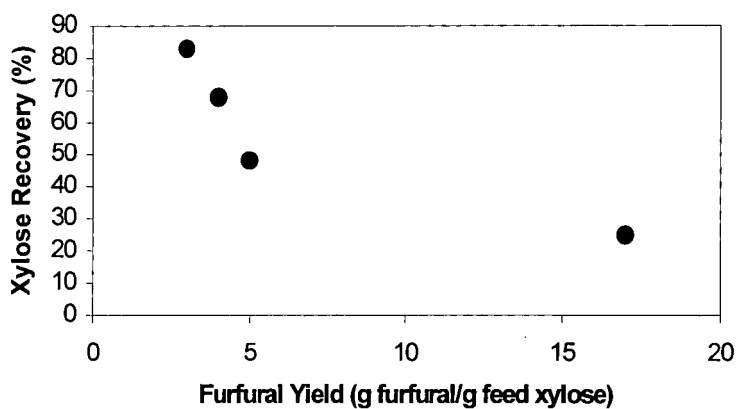


Figure 2: Xylose recovery as a function of furfural formation.